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Synthesis of Cycloprodigiosin Identifies the Natural Isolate as a Scalemic Mixture

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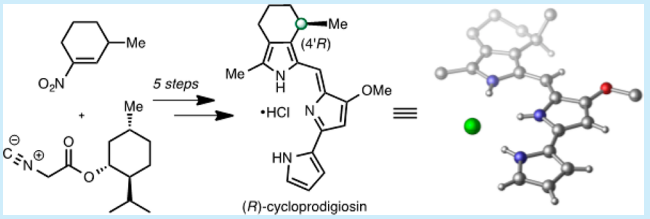
Supporting Information

ABSTRACT: The enantiomers of the natural product cycloprodigiosin were prepared using an expedient five-step synthetic sequence that takes advantage of a Schöllkopf–Barton–Zard (SBZ) pyrrole annulation with a chiral isocyanoacetate and a nitrocyclohexene derivative. Using chiral HPLC and X-ray crystallographic analyses of the synthetically prepared material and natural isolate (isolated from the marine bacterium *Pseudoalteromonas rubra*), naturally occurring cycloprodigiosin was determined to be a scalemic mixture occurring in an enantiomeric ratio of 83:17 (*R*)/(*S*) at C4′.



The prodigiosins are an intriguing class of compounds (Figure 1) characterized by a tripyrrolic core and

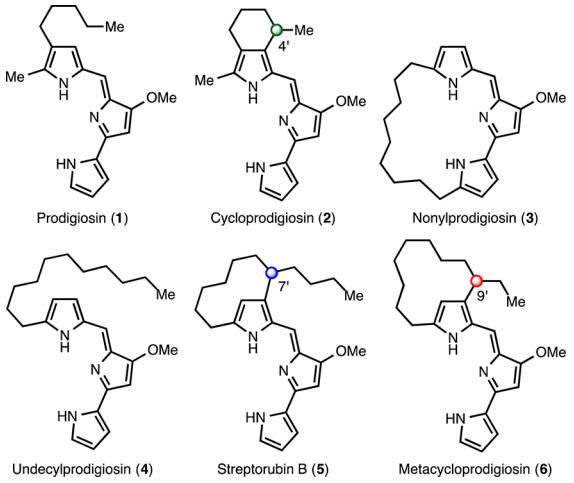


Figure 1. Representative members in the prodigiosin family of alkaloids.

remarkable bioactivity including anticancer, immunosuppressive, and antiparasitic properties.[1,2a−c] Since the first reports of their isolation in the 1960s, much effort has been expended in exploring their biosynthesis, structure, and bioactivity.[3a−c]

There is continued interest in the study of the prodigiosins because of their biological activity, which is believed to arise principally from the binding of ions by the tripyrrolic moiety[4a−c] as well as the interactions of these small molecules with B-cell lymphoma 2 (Bcl-2) proteins.[5] As recently advanced by Thomson,[6] it would appear that evolutionary selection of the cyclic prodiginines has been such as to maximize the ability of these molecules to bind ions and transport them across lipid bilayers. These favorable bioactivities have led to the development of a late-phase clinical candidate (Obatoclax) by Gemin X.[7,8] The exact mechanism of action for these molecules has yet to be elucidated. Since **2** is chiral, the stereochemistry at C4′ should have implications in the ability of each enantiomer to bind proteins, which may provide valuable insight into the mechanism of action of these small molecules.

In this Letter, we demonstrate using total synthesis and analysis of the natural isolate from *Pseudoalteromonas rubra* that natural cycloprodigiosin occurs as a scalemic mixture (83:17, (*R*)/(*S*)). Given the recent stereochemical elucidation of streptorubin B (**5**) by Challis in 2011 as a scalemic mixture, our finding suggests that the formation of **2** (analogous to the production of **5**) may involve a process (likely enzyme-mediated) that proceeds with imperfect enantioselectivity.[9]

The unusual combination of lipophilic and hydrophilic regions in the prodigiosins has not made their structural characterization straightforward. Indeed, even with the availability of modern analytical tools, a purported congener of streptorubin B, butylcycloheptylprodigiosin, has only very recently been correctly assigned.[6] Overall, however, the connectivity of the cyclic prodigiosins is now well established,

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which has paved the way for clearer insight into their biosynthesis.

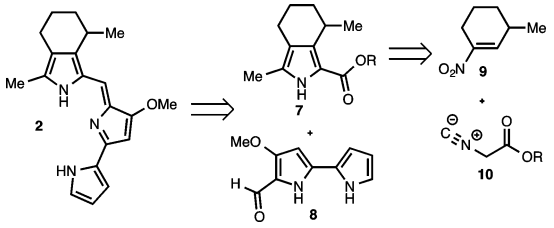
The biosynthesis of several of the cyclic prodigiosins (e.g., **5** and **6**) from their linear congener (**4**) has been shown to be mediated by a notable class of Rieske nonheme iron dependent oxygenases in *Streptomyces* spp.[10a,b,3c,11] The biosynthesis of cycloprodigiosin (**2**), first isolated from the marine bacterium *P. rubra*, presumably proceeds by cyclization of its linear congener prodigiosin (**1**). However, this hypothesis remains unsubstantiated. Additionally, while the constitution of cycloprodigiosin has been secured through careful analysis and total synthesis,[12a,b,13] it remains unknown whether cycloprodigiosin occurs in a racemic, scalemic, or enantiopure form. Insight into the nature of the existing stereocenter in **2** could have a significant impact on the understanding of its biogenesis as well as its mode of biological action.

A particularly intriguing feature of the cyclic prodigiosins is that naturally occurring streptorubin B is a scalemic mixture (95:5) at C7′ (see Figure 2) favoring the *S* enantiomer as established through studies by Challis and Thomson.[9,14,15] Strikingly, the predominant isomer of natural metacycloprodigiosin is 9′*R*; however whether **6** also occurs as a scalemic mixture has not been rigorously established.[10a] As a result, the stereochemistry at C4′ of **2** cannot be presumed by analogy to the other existing cyclic prodiginines. We sought to establish the absolute stereochemistry and enantiopurity of **2** through a comparison of synthetic material to the natural isolate.[10c,16]

Even though prior syntheses of **2** have been reported,[13,17] it was our goal to develop an efficient and scalable synthesis that would afford either enantiomer of **2** to facilitate our planned studies into the stereochemical assignment. Given the lack of information regarding the stereoconfiguration of the natural isolate of **2**, we designed a synthetic route that would provide access to the racemate as well as each enantiomer.

As shown in Scheme 1, we envisioned **2** arising from condensation of fused pyrrole **7** and bis-pyrrole aldehyde **8**

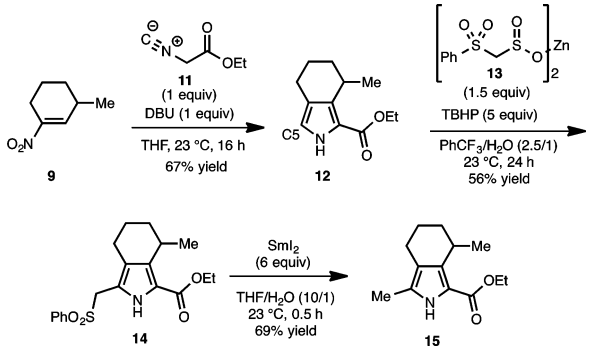
Scheme 1. Retrosynthetic Plan Highlighting a Two-Component SBZ Pyrrole Annulation



following the well-established precedent of Wasserman using HCl.[13] Key to this coupling would be an initial saponification/decarboxylation of the ester group of **7**. Fused pyrrole **7** could in turn be prepared from the two-component coupling of cyclic nitroalkene **9** and isocyanoacetates of general structure **10** using a Schöllkopf–Barton–Zard (SBZ) pyrrole synthesis.[18] In order to access both enantiomers of cycloprodigiosin, we planned to use a chiral auxiliary on the carboxy group of **10** (R = auxiliary) to facilitate separation of the diastereomers of **7** at a later stage in the synthesis.

The synthesis of racemic **2** commenced with the preparation of 3-methyl-1-nitrocyclohexene (**9**; see Scheme 2), which was obtained from 3-methylcyclohexene in one step according to the procedure of Estreicher and Corey.[19] Subjecting **9** to ethyl

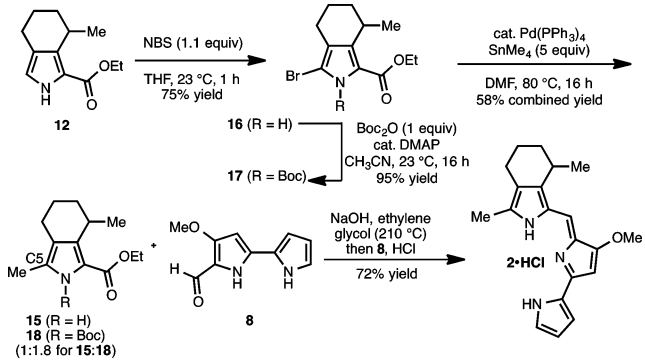
Scheme 2. Methylation Sequence Using Baran's PSMS Reagent (13)[20]



isocyanoacetate (**11**) effected an SBZ pyrrole annulation[18] to afford **12** in 67% yield. Several methods were then explored for installation of a methyl group at the pyrrole C5 position. Envisioning rapid access to methylated pyrrole **15**, we first investigated a method recently developed by Baran and co-workers.[20] Thus, treatment of pyrrole **12** with zinc bis[(phenylsulfonyl)methanesulfinate] (PSMS; **13**) and *tert*-butylhydroperoxide (TBHP) afforded methylenesulfone adduct **14** (56% yield), which upon treatment with freshly prepared samarium iodide was reduced to the desired C5 methylated pyrrole (**15**) in 69% yield. While this method provided efficient access to **15**, in our hands, an alternative approach proved to be more serviceable and cost-effective.

In this alternative sequence (Scheme 3), pyrrole **12** was brominated at C5 in 75% yield using NBS, which provided **16**

Scheme 3. Alternative Methylation Sequence Utilizing Brominated Pyrrole (17) as a Coupling Partner



in preparation for cross-coupling with a methyl group equivalent. As reported by Neumann and co-workers,[21] the coupling efficiency was significantly higher upon Boc-protection of the pyrrole nitrogen (to give **17**). A survey of Pd-catalyzed cross-coupling partners revealed that tetramethyltin serves as the most reliable coupling partner to provide a mixture of **18** and the deprotected analogue (**15**) in a combined 58% yield from **17**. Notably, the formation of both **15** and **18** is inconsequential as each compound serves as a substrate in the final condensation to form cycloprodigiosin. In the event, subjection of a mixture of **15** and **18** to NaOH in ethylene glycol at 210 °C (to effect a saponification/decarboxylation sequence) followed by the introduction of **8** yields racemic cycloprodigiosin·HCl (**2·HCl**) in 72% yield.[22]

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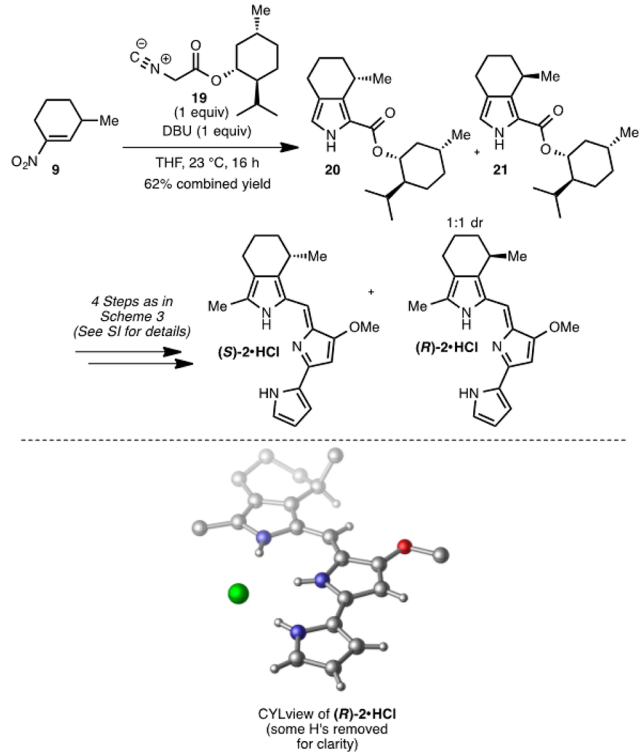
This synthetic sequence also provided the basis for the preparation of enantioenriched cycloprodigiosin using (−)-menthol-derived isocyanoacetate **19** (Scheme 4; prepared according to the procedure of Verkade)[23] in place of ethyl isocyanoacetate in the SBZ pyrrole annulation. The enantiomers of cycloprodigiosin ((*S*)-**2**·**HCl** and (*R*)-**2**·**HCl**) were prepared with similar efficiencies as described for the racemic case by advancing diastereomers **20** and **21** separately (see the Supporting Information for more details). X-ray crystallographic analysis of the HCl salt of enantiomer (*R*)-**2**·**HCl** has enabled absolute stereochemical characterization of the materials that we have prepared, which has set the stage for analysis of the natural material.

Scheme 4. Enantioselective Synthesis of *R*- and *S*-Cycloprodigiosin using (*R*)-Menthol Chiral Auxiliary (19)



A sample of naturally occurring cycloprodigiosin was obtained from *P. rubra* (Gauthier 1976, ATCC 29570) (see the Supporting Information for details) and purified by sequential normal and reversed-phase chromatography. In total, 0.2 mg of the natural product was obtained from 1 L of culture. With the natural isolate of cycloprodigiosin (from *P. rubra*) as well as our synthetic material in hand, we analyzed these materials using HPLC (Chiralpak IA) monitoring the absorbance at 500 nm.[12b,24] Comparison of the HPLC trace of the natural isolate to those of the racemic and enantiopure ((*S*)-**2** and (*R*)-**2**) material prepared by us is shown in Figure 2. (*R*)-**2** has a retention time of ∼5.43 min (Figure 2c) whereas (*S*)-**2** has a retention time of ∼10.02 min (Figure 2d). These observations are corroborated by our observations for racemic cycloprodigiosin (Figure 2a). The natural isolate displays two peaks that correspond to the two enantiomers in the ratio 83:17 for (*R*)-**2**/(*S*)-**2** (Figure 2b). Two independent production experiments gave identical enantiomeric ratios. Thus, it would appear that naturally occurring **2** is scalemic.

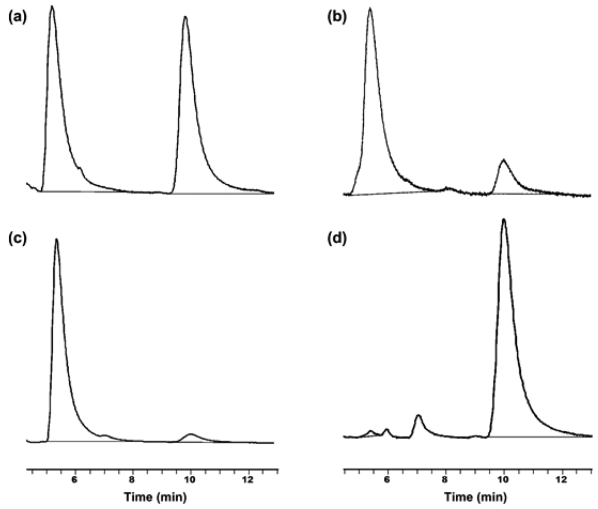


Figure 2. Chiral HPLC traces of (a) racemic cycloprodigiosin, (b) natural cycloprodigiosin, (c) synthetic (*R*)-cycloprodigiosin, and (d) synthetic (*S*)-cycloprodigiosin. See the Supporting Information for full HPLC traces.

These observations are consistent with those of Challis et al. for streptorubin B, albeit occurring with markedly lower enantiopurity in the case of cycloprodigiosin.

In conclusion, we report a rapid and novel synthesis of cycloprodigiosin that is easily modified to afford both enantiomers of the natural product. We have also achieved the growth and isolation of cycloprodigiosin from *P. rubra*. Our analyses of these materials using a combination of X-ray crystallography and chiral HPLC have revealed that naturally occurring cycloprodigiosin obtained from *P. rubra* occurs as a scalemic mixture, which is analogous to observations made previously for streptorubin B. These observations should have implications in terms of our understanding of the biosynthesis of cycloprodigiosin in *P. rubra*; biochemical studies are currently ongoing in our laboratory. Furthermore, these studies will inform investigations into the bioactivity of cycloprodigiosin, specifically in terms of the interactions of each enantiomer with biomolecules.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, chiral HPLC traces, X-ray crystallographic data, and NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01527.

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Notes

The authors declare no competing financial interest.

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Office of Biological and Environmental Research, through Contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy. We thank Dr. Antonio DiPasquale (UC Berkeley) for solving the crystal structure of **(*R*)-2·HCl** and determining its absolute stereochemistry (Supported by NIH Shared Instrumentation Grant S10-RR027172). We acknowledge the CYLView program (developed by Prof. Claude Y. Legault, Dept. of Chemistry, Université de Sherbrooke) for X-ray depictions.

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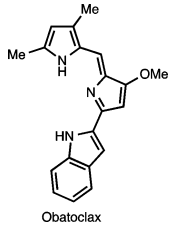

Obatoclax

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